

POLLEN EXINE MORPHOLOGY AND ITS ADAPTIVE SIGNIFICANCE¹

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The contribution of the research papers² presented in this symposium, "Systematics and Evolution of Pollen Characters in Angiosperms," could be summarized as the potential gift that new advances in electron microscopy can make to systematics.

I am reminded of an earlier stepping stone in palynological research by Professor G. Erdtman, who developed the acetylosis procedure, a technique that allowed the first concise and intimate view of pollen wall morphology. However much progress in this field we attribute to Professor Erdtman, he in turn told me some time ago that his inspiration in palynology had really been the outcome, the natural evolution, if you will, of the pioneering research of Dr. R. P. Wodehouse, whose drawings of stained pollen grains remain classic examples of what may be accomplished with a minimum of instrumentation and much dedication. This modesty was nevertheless characteristic of a great scientist whose impact on palynology is everlasting, and appropriately this symposium is dedicated to Professor Erdtman's memory.

Let me capsule the research papers presented to you this morning and add a few remarks of my own on potential adaptive significance of the diversity we have observed.

Recent research by Richard C. Keating (1973) on the Flacourtiaceae using only light microscopy has now been supplemented by both transmission and scanning electron microscopy, greatly enhancing observations of wall structure and grain surface. Keating considers the triaperturate

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² In sequence of presentation are:

Keating, R. C. 1975 [1976]. Trends of specialization in pollen of Flacourtiaceae with comparative observations of Cochlospermaceae and Bixaceae. *Grana* 15: 29-49.

Nowicke, J. W. 1975 [1976]. Pollen morphology in the order Centrospermae. *Grana* 15: 51-77.

Taylor, T. N. and D. A. Levin. 1975 [1976]. Pollen morphology of Polemoniaceae in relation to systematics and pollination systems: scanning electron microscopy. *Grana* 15: 91-112.

Tomb, A. S. 1975 [1976]. Pollen morphology in tribe Lactuceae (Compositae). *Grana* 15: 79-89.

Skvarla, J. J., B. L. Turner, V. C. Patel and A. S. Tomb. 1977. Pollen morphology in the Compositae and in morphologically related families. In V. H. Heywood (ed.) *Biology and Chemistry of the Compositae*. Academic Press, New York (in press).

grain, which is typical of many genera in the Flacourtiaceae, primitive. This opinion correlates well with vegetative and floral features, and thus places the family among the more primitive Angiosperms. Therefore, he assigns a basal position to the family in the Violales, for none of the other members of the order shows so primitive a pollen morphology or so little diversity. Of what variation does exist, however, Keating made the important observation that it is not unidirectional in all groups. For example, there is a tendency for near solid tecta to have been derived from finely reticulated tecta, as well as a tendency to form well developed infratectal spaces among both primitive and advanced taxa. These suggest non-homologous derivations and signal caution to the phylogenist who would base hypotheses on exine characters alone. Here, too, are examples of parallel evolution that again flash caution to those who would interpret like-morphologies invariably as having an immediate, common ancestry.

Keating outlined an interesting microporate tectal sequence in the Flacourtiaceae from those pollen having numerous micropores and flat tecta to those with micropores clumped into orifices between high ridged tecta. This condition masks a reticulated exine, for if it were true reticulation, the bacula would be organized below the ridges only, which is not the case. This sequence is valuable not only because it suggests an origin of reticulated exines from essentially tectate one, but it emphasizes the wide occurrence of micropores, a subject I shall return to shortly.

An undoubted feature of this symposium is the enormous scope of the papers, perhaps epitomized by the report of Joan W. Nowicke who characterized palynologically 16 families in the Centrospermae. This mass approach to pollen morphology is not one that I am criticizing. Quite the opposite, for I hope we can encourage such studies involving extensive research and synthesis of as many parts of as many organisms as we possibly can in our quest for an understanding of broad evolutionary pathways.

The report by Nowicke is, therefore, possibly a unique feat for sheer number of taxa involved, but it is also much more. For example, the finding of spinulose, perforated exines universally in the Centrospermae is perhaps as important a unifying character as the curved embryo or the unique sieve tube plastids now used to distinguish members of this order. Moreover, these new data do not preclude and perhaps even support the inclusion of the Caryophyllaceae and Molluginaceae, which otherwise do not possess betalains, with betalain-possessing families, and, thus, placing in the Centrospermae taxa having either anthocyanins or betalains.

Another significant contribution of her work is finding pollen grains in the Gyrostemonaceae and Achatocarpaceae distinct from those of the Phytolaccaceae. This confirms their separation from the latter, and questions their retention in the Centrospermae. In Nowicke's opinion, the pollen characters of *Theligonum* are sufficiently different from all other

Centrospermae known to suggest, in correlation with sporophytic features, its removal from the order, and perhaps, as suggested by Wunderlich (1971) placed in the Rubiaceae.

These more primitive families served as a springboard for the report by Thomas N. Taylor and Donald A. Levin who surveyed the more advanced Polemoniaceae. They found that grains with colporate and pantoporate apertures predominate, although tropical genera are typically pantoporate. Among former genera they observed a trend from large to small size pollen. Among those with pantoporate pollen, they noted an increase in aperture number with a decrease in pollen size from a tropical genus (*Bonplandia*) to one with strong temperate affinities (*Polemonium*). These and other data were compatible with a morphological and chromosomal phylogeny proposed by Verne Grant (1959). Some differences exist, however, and these need exploration.

Looking beyond comparative morphology, Taylor and Levin investigated the intriguing question of correlations between, for example, pollen size, which is so variable in the Polemoniaceae, and pollinator. They found a significant relationship between the large pollen transported by bats as well as between much smaller grains pollinated by various insects. They do not believe that these correlates are due to coadaptation directly, but rather to style length in which selection favors pollen size having enough metabolites for pollen tube growth to reach the embryo sac. However, we cannot dismiss the observation that a direct correlation may exist between the large, hexaploid pollen and large flowers of *Cobaea*, and bat pollination due to mechanisms as yet far from understood.

With regard to exine architecture, however, Taylor and Levin saw no apparent association with pollination mechanism and environmental parameters, a point I shall return to subsequently. They concluded that parallel evolution, at least in the Polemoniaceae, remains unidentified in pollen morphology, an interesting possibility in so variable a family palynologically.

The remainder of the symposium was devoted to the highly evolved and palynologically diverse Asteraceae. In his discussion of chicory exines (tribe Lactuceae), A. Spencer Tomb typified the wall structure as echinolophate, that is to say, with elaborate systems of ridges and spines disposed around and between apertures and pseudoapertures. This feature is the outstanding condition of the Lactuceae, for all subtribes recognized possess such grains. Some, but not all, subtribes also have echinate pollen, a much less elaborate spinulose pattern in which the spines are usually scattered over the surface and obvious gaps (lacunae) are absent. Tomb suggested subtribal modifications in classification based on these and other characters of the pollen correlated with morphological and chromosomal data. He also presented the probable evolutionary pathways for these pollen from echinate to echinolophate, from moderate-sized cavea to those exines

without a gap between the sexine and nexine, from tectate, perforate lacunal surfaces to nontectate lacunae, and from large sized grains to those having smaller sizes. These may be unidirectional trends, although some caution is necessary. Via polyploidy, for example, the more evolved, smaller grains could have independently given rise to much larger grains in various subtribes, and these, therefore, would be of more recent origin than the smaller, less ploid grains. Tomb also made this observation while discussing evolutionary direction involving the origin of echinolophate exines, namely, that less elaborate echinate exines may have evolved by reduction from more elaborate ones and that this atavism must not be confused with a single origin. In other words, the echinate condition can be both primitive and advanced.

The second paper on asteraceous pollen was presented by John J. Skvarla as a general survey of research on the tribe Inuleae, a tribe whose pollen exine is much less dramatically elaborate than that of the chicories, but none-the-less intriguing. He recognized two basic pollen types in the Inuleae, the first having simple or ramified bacula usually with abundant infrabaculate micropores (a type clearly related to the Senecioneae and Heliantheae), and the second in which the bacula are very complex having, for example, several levels of internal tecta, but lacking infrabaculate micropores and cavea. The second morphology is characteristic of the subtribe Tarchonantheae, as well as several genera scattered throughout the tribe (*Adenocaulon*, *Gymnarrhena*, *Osmites-Osmitopsis*), a morphology which the author notes is common among the tribe Anthemideae. As a result of this study, Skvarla suggests that much of the infratribal classification of the Inuleae is artificial.

We have observed among these surveys, involving largely comparative morphology of the exine, the substantial value of palynology in systematic studies. Clearly, palynological evidence may be useful in placing taxa of uncertain affinity, of suggesting taxonomic rearrangement, as well as corroborating other lines of evidence in order to establish evolutionary hypotheses.

Exine diversity is extraordinary in the sample we have just observed, illustrating surely the tremendous competence of flowering plants in designing and building these walls. But why, we might ask, have these plants bothered? Are we to assume that what we have observed are but meaningless manifestations; if not, can we recognize that there are adaptive elements at play and that diversity has resulted from selective forces (Heslop-Harrison 1971)? Clues do exist of the adaptive value of pollen wall structure. Basically the wall serves to protect contents from desiccation, it facilitates or permits efficient germination, and it can also be expected to have adaptive roles concerned with dispersal. In relation to these functions, let us examine the most conspicuous feature of the wall so universally stressed during this symposium, the aperture.

Apertures form the preferred paths of exit for the pollen tube, but their function extends beyond providing an escape route. For example, they act as regulators controlling the gain and loss of water. Very briefly, the plates, plugs and granules which cover many apertures consist of typically impermeable exine which can be observed to close on dehydration caused by an infolding of the intine at the aperture site. Imbibing water, however, brings about a gaping of the aperture with the exine being carried apart on the surface of the extended intine or left at the aperture margins. A further aid to this regulation is the so-called pollenkit or lipid of tapetal origin which on desiccation forms a coating over the apertures effectively obstructing water loss. As regulatory devices, this apertural system seems simple enough, but they do illustrate regulatory functions that have adaptive values and are therefore likely to have evolved under the pressure of selection.

A conspicuous feature of apertures is their numerical difference. Why, for example, has the polyaperturate condition apparently evolved from the more common triaperturate dicotyledonous pollen? Could aperture number itself be adaptive? If so, there must be functions other than providing for the exit of a single pollen tube. One function is certainly related to the release of enzymes and other proteins from moistened grains to stigmatic surfaces. Among these proteins emitted from the apertural intine are lytic enzymes, suggesting a function associated with the digestion of substrate on the stigma and thus with the initial nutrition of the tube. Other substances are concerned with compatibility reactions which determine what pollinations will proceed to fertilization and the next generation and which ones will not. They are recognition substances which may or may not be recognized by sites on the protein pellicle of stigmatic papillae (Mattsson et al. 1974). Therefore, aperture adaptation is not only related to germination, but to the control of the breeding system and the physiology of the emerging tube as well. When each aperture is seen as a site for storage and release of physiologically significant materials, and not just an area for potential pollen tube exit, this places the evolution of aperture number in a new perspective.

What of those parts of the exine beyond the apertures? Early observations by Wodehouse (1935) are still appropriate: pollen grains from those plants having reverted to anemophily differ from those of their related entomophilous species by possessing grains with thinner and smoother exines. For example, grains of anemophilous *Populus* are smooth compared to the reticulated exines of *Salix*, a genus largely of entomophilous species. Or, on discussing the wide differences in pollen encountered in the tribe Anthemideae (Asteraceae), Wodehouse noted that the insect pollinated species of *Anthemis* (*Leucanthemum*) and *Chrysanthemum* have grains with well developed spines and thick exines, whereas those of *Artemisia*, *Crossostephium* and *Picrothamnus*, which are wind pollinated,

have grains in which the spines are vestigial or absent and the exines are much less thick. He concluded that members of this tribe may be divided rather sharply into two groups consisting of those which are echinate grained and insect pollinated and those with smooth or nearly smooth grained and wind pollinated.

There are also additional characteristics of pollen correlated with mode of pollination. Clearly the lipoidal component of the exine differs, for it is absent or present in only small amounts in anemophilous species, while being a most conspicuous adjunct of the pollen of entomophilous plants. This pollenkit provides not only much of the visual and olfactory stimulus needed to guide insects, but it also provides the means for affixing the grains to the pollinator's body (Heslop-Harrison 1971).

But what of the elaborate architectural detail of the sexine which I should mention is largely of sporophytic, not gametophytic, origin? Proteinaceous material can now be recognized in the intra- and interbacular spaces of the wall as well as in the cavea between the sexine and the nexine among asteraceous pollen. This material is entirely of sporophytic origin, having been injected into the cavities of the sexine following derivation from membrane-bounded cisternae released from the tapetum of the anthers during its dissolution (Heslop-Harrison et al. 1973). On moistening, these sexinous-held sporophytic proteins are discharged by the pollen through the micropores in the tectum, micropores which we have observed as a very common feature among the diverse families observed today. This discharge has been particularly well demonstrated by its release within 5 to 30 seconds following pollen moistening of numerous antigens and allergens from the micropores at the base of spines of *Ambrosia* and *Cosmos* (Knox and Heslop-Harrison 1971, Howlett et al. 1973). Therefore, there is a functional significance to the complex internal architecture of the tectal and baculate layers of the exine: it is basically a storage site for materials derived from the tapetum which, through micropores, provides passage for injection of stored materials and for their release when pollen is moistened. In nontectate exines, the spaces between the muri are also concerned with the storage of tapetum derived materials. In the Asteraceae, the exine storage capacity is further enhanced by the development of cavea, which provide enormous reservoirs for products transferred from the sporophyte.

The materials undoubtedly function in several ways. They possess a role in inter- and infraspecific incompatibility systems where the genetic control resides with the parental sporophyte (Heslop-Harrison 1967). This has been shown for *Populus* (Knox et al. 1972) where proteins from the pollen of one species have been used to make pollen of an incompatible foreign species acceptable, and in another example for *Cosmos bipinnatus* (Howlett et al. 1973), extracts of compatible pollen make self-incompatible pollen fully self-pollen acceptable by the stigmas. In addition, exine-held fractions

have recently been shown associated with incompatibility responses in the Brassicaceae (Heslop-Harrison et al. 1974). It seems clear, that for a wide range of flowering plants the exine is the site of recognition materials in incompatibility systems. I should note that the allergenic response in man to these outer wall-based proteins, as well as those held by the intine, is no more than an unfortunate accident of evolution, for their role is to act as recognition substances during pollen-stigma interactions, not to harass those among us who produce immunoglobulin E on contact followed by alarming allergic symptoms.

Briefly, we have glimpsed at several, newly discovered roles involving the pollen wall, largely thanks to the research of Professor J. Heslop-Harrison and his team at the Royal Botanic Gardens, Kew, and to Professor John Rowley and his associates at the Palynological Laboratory, Stockholm. These functional roles of pollen can be related to a number of morphological characters only recently exposed clearly by electron microscopes. For example, the widespread asteraceous cavea could be considered adaptively important, as indeed could the frequency, size and position of the micropores, and the diverse baculate spaces of the sexine. All relate to the storage and conveyance of significant proteinaceous compounds from one generation to the next, yet before the advent of electron microscopy, these features were virtually or entirely unobservable. We need more observations and experiments before the full significance of exine sculpturing is realized. It is now apparent that such diversity is not all incidental, rather, that it is more likely to be accidentally interwoven with adaptive elements which has given us a highly intricate and in many cases selected covering to the male gametophyte of higher plants. More physio-morphological research similar to that described today and taking place at Kew and in Stockholm is imperative before the relevance of this minute plant, the pollen grain, is fully understood and the selective advantages of its innumerable diversities are fully appreciated.

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